Genetic polymorphisms in heterocyclic amine metabolism and risk of colorectal adenomas

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High red meat intake has been linked with an increased risk of colorectal cancer and adenomas. During high temperature cooking of red meats, heterocyclic amines (HCAs) are generated; however, to be carcinogenic, they must be metabolized by enzymes including cytochrome P450 1A2 (CYP1A2) and *N*-acetyltransferase 1 (NAT1) and/or *N*-acetyltransferase 2 (NAT2). We have conducted a clinic-based case–control study of colorectal adenomas that focused on assessment of exposure to HCAs (estimated by use of a HCA database and meat cooking module) and modification of these exposures by genetic factors. We have previously reported that intake of MeIQx was associated with an increased risk of colorectal adenomas [overall association at 80th percentile, > 27.00 ng/day: odds ratio (OR) = 2.68, 95% confidence interval (CI) 1.58–4.55]. Here, we report our evaluation of whether variation in CYP1A2, NAT1 and/or NAT2 modify the association between HCAs and colorectal adenoma formation in 146 cases and 228 frequency-matched controls. The *NAT1*10* allele was associated with a nonsignificant increased risk of colorectal adenomas (OR = 1.43; 95% CI 0.86–2.36). Further, when we analysed 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx) intake as a categorical variable, we observed a six-fold increase in adenoma risk among rapid NAT1 acetylators who consumed more than 27 ng a day (OR = 6.50; 95% CI 2.16–19.7), whereas among slow NAT1 acetylators, the increase in risk was two-fold (OR = 2.32; 95% CI 1.12–4.81). While suggestive, the results were not significantly different from each other on either an additive or multiplicative scale. In contrast, *NAT2* genotype and CYP1A2 and NAT2 hepatic activity measured by caffeine urinary metabolites were not associated with adenoma risk, although an increase in risk with rapid CYP1A2 activity could not be ruled out (OR = 1.46; 95% CI 0.76–2.81). Moreover, there was no evidence that the effect of MeIQx was enhanced among subjects in any subgroup defined by variation in these measures. These results are compatible with the hypothesis that high HCA exposure is associated with an increased risk of colorectal adenomas, particularly in genetically susceptible subgroups. Further study of larger populations is needed to confirm and extend these observations. *Pharmacogenetics* 12:145–150 © 2002 Lippincott Williams & Wilkins

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Introduction

High red meat intake is suggested to be associated with an increased risk of colorectal cancer [1,2], and with adenomatous polyps [3–7]. A major class of carcinogens generated during high temperature cooking of red meats is the heterocyclic amines [8]. Although DNA adducts of HCAs have been detected in human colonic tissue [9], it is unclear whether HCAs, such as 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx) and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), induce gastrointestinal tumours in humans. Using surrogates of exposure to these carcinogens, epidemiological studies have produced suggestive, but inconsistent evidence for a link to colorectal cancer [1,10–15] and colorectal adenoma formation [15,16].

For HCAs to be carcinogenic, they must be metabolized by enzymes that include cytochrome P450 1A2 (CYP1A2) [17], *N*-acetyltransferase 1 (NAT1) and/or *N*-acetyltransferase 2 (NAT2) [18], and those in the sulfotransferase (SULT1A1, SULT1A3, SULT1E1 and SULT2A1) family [19–22]. Each of these enzymes exhibits genetic polymorphisms in humans [23–25], although the genetic polymorphisms identified in

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CYP1A2 have not been correlated with the metabolic variability observed [26–28]. Rapid acetylator phenotype has been reported to be associated with susceptibility to colorectal cancer [17,29,30], but not with colorectal polyp risk [17,31]. However, studies that classified acetylator status using genotypic techniques have not shown a consistent overall association with either endpoint [32–38].

One study that assessed phenotypic expression of CYP1A2 in combination with NAT2 acetylation status reported a higher frequency of colorectal cancer and polyp cases who were rapid for both enzymes compared with controls [17]. In addition, the authors reported that individuals with this combined rapid–rapid phenotype were at particular risk among subjects who preferred well-done meat. The possible modifying effect of genetic polymorphism in *NAT1* was not assessed. Other case–control studies have also observed a significant association of high red meat intake with colorectal adenoma [31] and colorectal cancer [32] that was limited to *NAT2* rapid acetylators, while a further study did not [39].

We recently reported results from this population of military officers suggesting carcinogenic compounds formed by high-temperature cooking techniques may be positively associated with colorectal adenoma development [40]. In this study, we assessed the role of *CYP1A2*, *NAT1* and/or *NAT2* in colorectal adenoma formation and whether they modify the previously reported HCA association.

Materials and methods

We conducted a case–control study of colorectal adenomas to investigate the role of HCAs and genetic susceptibility in a medical centre serving mainly active and retired military officers and their families who have been described previously [16]. Briefly, the cases comprised patients who were diagnosed with colorectal adenomas at sigmoidoscopy or colonoscopy and controls who were selected among subjects without colorectal adenomas at sigmoidoscopy. Although 10% of control subjects were referred to the clinic for sigmoidoscopy because of gastrointestinal symptoms, such as blood in stool or diarrhoea, the majority were screened to meet military requirements. Excluding the control subjects with gastrointestinal symptoms from the analysis did not alter the findings. The 146 colorectal adenoma cases in this report were frequency-matched by gender and age in 5-year intervals to 228 control subjects. Cases who reported a previous adenoma were excluded from the study. Blood was collected from both cases and control subjects during the clinic visit. A self-administered food frequency questionnaire (FFQ), an overnight urine collection kit and a urine caffeine collection kit with cooler and ice packs were delivered to each subject's home.

A meat cooking module including 23 meat items, doneness level and cooking method, was also completed by the subjects. We estimated HCA intake using an HCA database that we developed [41,42] and the response from the FFQ. First, we estimated gram consumption of each meat item (steak, hamburger patty, pork chops, etc.), using frequency and portion size, by cooking technique and doneness level. Then we derived HCA intake by multiplying grams of meat by HCA concentration measured for each cooking technique/doneness level contribution for that meat type. HCA concentration was summed across all of the meat items.

Genotyping of *NAT2* was determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) on DNA samples using a previously described method [43,44]. Six *NAT2* slow acetylator alleles were ascertained: *NAT2*5A*, *NAT2*5B*, *NAT2*5C*, *NAT2*6*, *NAT2*7* and *NAT2*14*. Genotyping of *NAT1* was also determined by PCR-RFLP on DNA samples [45]. Nine *NAT1* acetylator alleles (*NAT1*3*, *NAT1*4*, *NAT1*10*, *NAT1*11*, *NAT1*14A*, *NAT1*14B*, *NAT1*15*, *NAT1*17* and *NAT1*22)* and 17 *NAT1* genotypes were identified. Individuals were classified as rapid acetylators if they possessed at least one *NAT1*10* allele [46,47]. Due to the low frequency of slow acetylators, all *NAT1* genotypes other than those possessing the *NAT1*10* allele were combined to form the reference group. In addition, subjects were phenotyped for CYP1A2 and NAT2 activity by measuring urinary caffeine metabolites following methods detailed elsewhere [48]. Out of 374 subjects, 351, 351, 318 and 350 samples were assayed for *NAT2* genotype, NAT2 phenotype, *NAT1* genotype and CYP1A2 phenotype, respectively. Missing laboratory data were primarily due to inadequate sample availability, DNA degradation, or failure in the experimental assay. Laboratory personnel were blinded to case status for all assays.

Unconditional logistic regression was used to calculate odds ratios (OR) and 95% confidence intervals (CI) between HCA intake, genetic polymorphisms and colorectal adenoma risk. Odds ratios for PhIP and MeIQx are presented in 10 ng/day increments as well as categorically, in quintiles, according to the HCA distribution in the control subjects. Interaction between polymorphisms and HCA intake on colorectal adenoma risk was assessed on both the multiplicative and additive scale. Interaction was evaluated by creating indicator variables for the combination of metabolic activity (i.e. slow vs rapid) and HCA intake levels, with slow metabolizers with low intake serving as the reference category. *P*-values for the test for multi-

plicative interaction were calculated by the likelihood ratio test, comparing the above model with a model containing indicator terms for the main effect of genotype and HCA exposure only. The 80th percentile of MeIQx intake ($\leqslant$ 27.00 ng/day or $>$ 27.00 ng/day) and 60th percentile of PhIP intake ($<$ 63.00 ng/day or $\geqslant$ 63.00 ng/day), respectively, were used as the cut-off for these calculations [40] since the excess risk of colorectal adenomas was confined to the fifth quintile for the former and the upper two quintiles for the latter. Similar results were observed when the interaction was assessed using the 80th percentile cutoff for PhIP ($<$ 140 ng/day or $\geqslant$ 140 ng/day; data not shown). An additive interaction was evaluated by testing for significance of the estimated excess risk for interaction [49]. All ORs were adjusted for age, gender, pack–years of cigarette smoking, total caloric intake, physical activity, nonsteroidal anti-inflammatory drug use, dietary fibre intake and reason for screening (routine or other).

Results

Eighty-six percent of the cases and 89% of the control subjects were of Caucasian origin, and the median age of the cases and control subjects was 58 and 59 years, respectively. Details on their demographics and risk factors have been reported previously [16].

High intake of PhIP and MeIQx were associated with increased colorectal cancer risk [40]; however, neither of the *NAT* genetic polymorphisms was associated with colorectal adenoma risk (Table 1), although there was some evidence of an increased risk for individuals who were genotypically rapid for *NAT1*. Similarly, greater CYP1A2 and NAT2 phenotypic expression were not independently associated with colorectal adenoma risk. Taking both phenotypes into consideration, simultaneously, did not alter these findings (data not shown).

Potential effect modification of HCA exposure and colorectal adenoma risk by NAT2 or CYP1A2 phenotypes were explored. No effect modification by either of the phenotypes was observed when the colorectal adenoma risk associated with MeIQx consumption was assessed (data not shown). Results were similar when *NAT2* genotype was used as the possible modifier. When the NAT2 and CYP1A2 phenotypes were combined, no increase in colorectal adenoma risk was observed among individuals with the rapid–rapid phenotypes.

In contrast, an increase in colorectal adenoma risk associated with MeIQx consumption was observed in subjects who were genotypically rapid for *NAT1*. When MeIQx intake was analysed as a continuous variable, a 34% increase in colorectal adenoma risk per 10 ng of MeIQx intake (OR = 1.34; 95% CI 1.08–1.66) was observed among rapid *NAT1* acetylators, whereas among slow *NAT1* acetylators, a 12% increase in risk was observed (OR = 1.12; 95% CI 0.98–1.27). Similarly, when we analysed MeIQx as a categorical variable, we observed a six-fold increase in adenoma risk among rapid *NAT1* acetylators, those who consumed more than 27 ng a day (OR = 6.50; 95% CI 2.16–19.6) compared with a two-fold increase in risk among slow acetylators (OR = 2.32; 95% CI 1.12–4.81). Analysing the data using a joint effects model also resulted in similar associations (Table 2) suggesting that MeIQx may be associated with a greater risk of colorectal adenoma formation among individuals with the *NAT1*10* allele.

Table 1 Odds ratios (OR) and 95% confidence intervals (CI) between genotype or phenotype and colorectal adenoma risk

Genotype	Case	Control	OR (95% CI) adjusted[1]
NAT1[2]			
No *NAT1*10*	77 (58.3%)	126 (65.6%)	1.00 (ref)
Rapid	55 (41.7%)	66 (34.4%)	1.43 (0.86–2.36)
NAT2[3]			
Slow	79 (55.2%)	110 (52.9%)	1.00 (ref)
Rapid	64 (44.8%)	98 (47.1%)	0.91 (0.57–1.45)
Phenotype[4]	Median (range)	Median (range)	
CYP1A2	6.47 (0.8–34.8)	5.49 (0–32.3)	1.02 (0.97–1.07)
Slow ($\leqslant$ 12)	114 (81.4%)	183 (87.1%)	1.00 (ref)
Rapid ($>$ 12)	26 (18.6%)	27 (12.9%)	1.46 (0.76–2.81)
NAT2	0.43 (0–4.45)	0.6 (0.1–4.12)	0.97 (0.75–1.23)
Slow ($\leqslant$ 0.6)	75 (53.6%)	105 (49.8%)	1.00 (ref)
Rapid ($>$ 0.6)	65 (46.4%)	106 (50.2%)	0.86 (0.54–1.38)

[1] Odds ratios adjusted for age, gender, total caloric intake, fibre intake, reason for screening, physical activity, pack–years of cigarette smoking, and use of non-steroidal anti-inflammatory drugs (NSAIDS). [2] Individuals were classified as rapid acetylators if they possessed at least one *NAT1*10* allele. Individuals were classified as slow acetylators if they possessed at least one *NAT1*14, NAT1*15, NAT1*17* or *NAT1*22* allele. Due to the low frequency of slow acetylators, all *NAT1* genotypes other those possessing the *NAT1*10* allele (rapid) were combined to form the reference group. [3] Individuals were classified as slow acetylators if they had two slow acetylator alleles, and rapid acetylators if they had at least one *NAT2*4* or *NAT2*12* allele. [4] Urinary molar ratio of caffeine metabolites [(17X + 17U)/137X] was used as an index for CYP1A2 enzyme activity; ratio of AFMU:1X was used to assign NAT2 acetylation phenotype.

Table 2 **Odds ratios and 95% confidence intervals of the joint association between HCA exposure and *NAT1* genotype and colorectal adenoma risk**

NAT1 genotype[1]	MeIQx (80th percentile)	Cases	Controls	Adjusted (95% CI)[2]
	⩽ 27.00 ng	78	160	1.00 (ref)
	> 27.00 ng	54	32	2.68 (1.58–4.55)
No *NAT1*10*	⩽ 27.00 ng	45	101	1.00 (ref)
No *NAT1*10*	> 27.00 ng	32	25	2.44 (1.20–4.99)
Rapid	⩽ 27.00 ng	33	59	1.23 (0.67–2.24)
Rapid	> 27.00 ng	22	7	7.67 (2.77–21.3)[3]
Multiplicative interaction				2.56 (0.73–9.02)
Additive interaction				5.00 (−2.53, 12.5)
	PhIP (60th percentile)			
	< 63.00 ng	57	115	1.00 (ref)
	⩾ 63.00 ng	75	77	1.93 (1.17–3.18)
No *NAT1*10*	< 63.00 ng	31	77	1.00 (ref)
No *NAT1*10*	⩾ 63.00 ng	46	49	2.17 (1.13–4.14)
Rapid	< 63.00 ng	26	38	1.66 (0.81–3.39)
Rapid	⩾ 63.00 ng	29	28	2.81 (1.33–5.92)
Multiplicative interaction				0.78 (0.28–2.18)
Additive interaction				−0.02 (−2.18, 2.14)

[1]Individuals were classified as rapid acetylators if they possessed at least one *Nf* they possessed at least one *NAT1*10* allele. Due to the low frequency of slow acetylators, all *NAT1* genotypes other than those possessing the *NAT1*10* allele were combined to form the reference group. [2]All odds ratios adjusted for age, gender, total calorific intake, fibre intake, reason for screening, physical activity, pack–years of cigarette smoking, and use of non-steroidal anti-inflammatory drugs (NSAIDs). [3]Subgroup results presented in the text can be approximated from this table. The six-fold increase in risk observed among rapid *NAT1* acetylators who consumed more than 27 ng a day of MeIQx is approximately 7.67 divided by 1.23.

No enhanced increase in risk among rapid *NAT1* acetylators who were exposed at higher levels of daily PhIP consumption (⩾ 63.00 ng) was observed (OR = 1.56; 95% CI 0.69–3.54).

Effect modification of the association between *NAT1*10* allele and colorectal adenoma by MeIQx intake level was also assessed. No association between colorectal adenoma risk and the *NAT1*10* allele was observed among subjects who consumed less than 27 ng of MeIQx per day (OR = 1.21; 95% CI 0.65–2.23). In contrast, an increase in adenoma risk with the *NAT1*10* allele was noted among subjects with daily consumption greater than 27 ng of MeIQx (OR = 2.86; 95% CI 0.93–8.79), although this association did not reach statistical significance.

Discussion

Despite considerable research into delineating the mechanism involved in the consistent association observed between high red meat consumption and colorectal cancer, it is still largely unknown as to which HCAs in red meat, if any, and which biotransformation enzymes are involved in this carcinogenic process. In this study, MeIQx intake appeared to be associated with increased risk of colorectal adenomas, particularly at higher intakes and in *NAT1* rapid acetylators. In contrast, no evidence of an effect modification by *NAT2* or *CYP1A2* was observed.

The data reported here are broadly consistent with a recent animal study [50] and with data of Chen *et al.* [51]. A greater dose-dependent increase in PhIP-induced aberrant crypt foci in rapid acetylator rats compared with slow acetylator rats was observed in the former, and a stronger association of red meat intake and colorectal cancer was observed in men who had the rapid acetylation genotype of *NAT1* and *NAT2* in the latter. Although we also observed an increase in colorectal adenoma risk among rapid acetylators with higher HCA consumption, the risk was limited to *NAT1* rapid acetylators with higher MeIQx consumption. Furthermore, we did not observe a metabolic effect of high CYP1A2 activity as has been reported previously [17,52–54].

The strength of this study is that it was designed to evaluate the interrelationships between genetic polymorphisms in metabolizing enzymes (i.e. CYP1A2 phenotype, *NAT2* genotype and phenotype, and *NAT1* genotype) and specific HCAs (i.e. MeIQx, PhIP) postulated to be involved in HCA metabolism in colorectal adenoma formation. Furthermore, the outcome of interest was pre-cancerous adenoma, which should not have influenced their responses about usual dietary habits as a cancer diagnosis may. However, the fact that we examined multiple potential interactions between these genetic polymorphisms and HCA intake suggests that these results should be interpreted with caution. Furthermore, other enzymes that may be involved in HCA metabolism, such as the sulfotransferases have not been accounted for.

The results in this study are compatible with the hypothesis that high exposure of heterocyclic amines is a modifiable cause of colorectal adenoma, particularly

in a subpopulation of genetically susceptible individuals (i.e. *NAT1* rapid acetylators). This gene–environment interaction could potentially be important in populations in such as Asians [55,56] and African-Americans [45] in which the rapid acetylator allele frequency is high. With the dietary changes associated with a more Western lifestyle in Asian populations, an increase in colorectal cancer incidence may be observed [54,57,58]. Further study is clearly warranted to confirm and extend these observations, particularly in populations with higher rapid acetylation allele frequencies.

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